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Tissue Processing, Staining And Image Processing Of Pathological Cancer Images: A Review

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Abstract— Digital image processing enables synthesis of images for characterisation of properties. Image segmentation is the most critical functions in image analysis and processing. Fundamentally segmentation results affect all the subsequent processes of image analysis such as object representation and description, feature measurement, and higher level tasks such as object classification.Cancer, at early stage, detection can be possible only with micro image processing. Processing and staining of Cancer tissues for pathological examination through micro images is a difficult task. The study intends to compare the set of image segmentation and edge detection algorithms that can be employed in the image segmentation process.Review of current methodologies of image segmentation using automated algorithms, which are accurate and require little user interaction, is possible especially for pathological medical images. In this paper we project the study of processing, straining and capturing of the pathological images for processing to detect cancer early.

Index Terms—Image processing, Pathological Images, edge detection, Image segmentation, Tissue processing, staining, cancer.

I. INTRODUCTION

Medical images play vital role in assisting health care providers in proper diagnosis and correct treatment. Availability of pathologist in the remote areas is less to study the pathology images such as cancer images. Digital image processing techniques can identify the features more accurately and provide the appropriate status on disease. Consequently the use of computer-aided systems becomes very essential to overcome these limitations.

A. Some General Rules for the biopsy Procedure [4-6]:

1. The larger the lesion, the more the biopsies that should be taken from it because of the variability in the pattern that may exists and the fact that the diagnostic areas may be present only focally.
2. In ulcerated colour, biopsies of the central ulcerated areas may show only necrosis and inflammation. Biopsies should be taken from the periphery that includes normal and diseased tissue.
3. The biopsies should be deep enough that the relationship between colour and stroma can be properly assessed.
4. Deeply seated lesions are sometimes accompanied by a prominent peripheral tissue reaction which may be characterized by chronic inflammation, hyperaemia, fibrosis, calcification and metastatic bone formation. If the biopsy is too peripheral, this may be the only tissue obtained.
5. When several fragments of tissue are obtained they should be sent to the pathology laboratory and all of them submitted for microscopic examination.
6. Crushing or squeezing of the tissue with forceps should be carefully avoided.
7. Once the biopsy is obtained, it should be placed immediately into container with adequate volume of fixative.

B. Tissue Processing:

In order to cut thin sections of the tissues, it should have suitable hardness and consistency when presented to the knife edge. These properties can be impacted by infiltrating and surrounding the tissue with paraffin wax, colloidin or low viscosity nitrocellulose, various types of resins or by freezing. This process is called tissue processing. It is done in stages. It can be subdivided into dehydration, clearing, impregnating and embedding. It is important that all specimens are properly labelled before processing is started. For labelling, pen containing ordinary ink should not be used. Printed, graphite pencil written, type-written or India ink written labels are satisfactory.

A system of transportation is required to carry the tissue through various steps in processing. The cut specimens are put in muslin cloth together with their labels and are then transported from reagent to reagent in metal containers that have perforated walls, so that the reagent enters into the tissues. Tissue processing is a long procedure and requires 24 hours.

C. Staining:

Staining is a process by which we give colour to a section. There are hundreds of stains available.

Generally the stains are classified as:

i. Acid stains
ii. Basic stains
iii. Neutral stains

All dyes are composed of acid and basic components. Dye is a compound which can colour fibres and tissue constituents.

Acid Dyes: In an acid dye, the basic component is coloured and the acid component is colourless. Acid dyes stain basic components e.g. eosin stains cytoplasm. The colour imparted is shade of red.

Basic Dyes: In a basic dye the acid component is coloured and the basic component is colourless. Basic dyes stain acidic components e.g. basic fuchsine stains nucleus. The colour imparted is shade of blue.

Neutral Dyes: When an acid dye is combined with a basic dye a neutral dye is formed. As it contains both coloured radicals, it gives different colours to cytoplasm and nucleus simultaneously. This is the basis of Leishman stain.

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II. REVIEW

In the current practice of medicine, histo-pathological examination of biopsies is the most commonly used method to locate and classify diseases including cancer. In cancer diagnosis, pathologists visually examine the changes in cell morphology and tissue distribution under a microscope and determine whether a biopsy contains any malignant (cancerous) region and, if so, the cancer type and its malignancy level (grade). However, as it mainly relies on the visual interpretation, this examination may lead to a considerable amount of subjectivity, especially in cancer grading [14, 15]. To reduce this subjectivity, it has been proposed to use computational methods that rely on the quantification of a tissue by defining mathematical features [16–20]. Although the very first step in this quantification is the segmentation of a tissue image into homogeneous regions, these studies have not mainly focused on this problem and have extracted features from the tissue image assuming that it is homogeneous. Nevertheless, besides having many heterogeneous regions in a tissue image, the existence of such regions and their ratio help pathologists determine the cancer grade.

It has been proposed to segment an image into homogeneous regions by either connecting adjacent pixels or locating edges according to a homogeneity measure. Numerous studies have defined the homogeneity based on the colour information of pixels and/or the spatial relations between the colours (i.e., texture information of pixels) [21,22]. A large subset of them quantizes the pixels of an image into clusters using the colour information alone and considers the connected pixels of the same cluster as a homogeneous region [23–28]. One common method to find such clusters is to employ the colour histogram of the image.

For example, Park et al. [24] perform morphological operators on the histogram, while Shafarenko et al. [25] apply the watershed algorithm to the histogram to detect the clusters. Besides employing the colour histogram, different approaches (such as fuzzy [26, 27] and genetic [28] approaches are also used to obtain the clusters. Another subset of studies proposes to use spatial information of pixels in addition to their colour information [29–32]. The colour and the texture information could be used consecutively or together in segmentation. For example, the JSEG algorithm proposed by Deng and Manjunath [30] first uses pixel colour information to quantize them into clusters, without considering their spatial relations. Following the quantization, they define a homogeneity criterion to quantify the texture of the colour quantized pixels. In contrast, in the algorithm proposed by Chen et al. [32], the texture is extracted from the gray-scale of the image and it is used together with the colour information to obtain the overall segmentation.

The review of the research on various research methodologies applied for image segmentation and various research issues in this field of study. This study aims to provide a simple guide to the researcher for those carried out their research study in the image segmentation

Image segmentation has a promising future as the universal segmentation algorithm and has become the focus of contemporary research. In spite of several decades of research up to now to the knowledge of authors, there is no universally accepted method for image segmentation, as the result of image segmentation is affected by lots of factors, such as: homogeneity of images, spatial characteristics of the image continuity, texture, image content. Thus there is no single method which can be considered good for neither all type of images nor all methods equally good for a particular type of image. Due to all above factors, image segmentation remains a challenging problem in image processing and computer vision and is still a pending problem in the world.

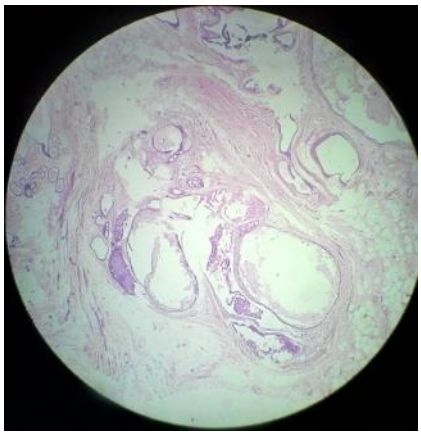

Figure-1.1: Fibrocystic disease (FCD) with 4X magnification of the breast cancer image

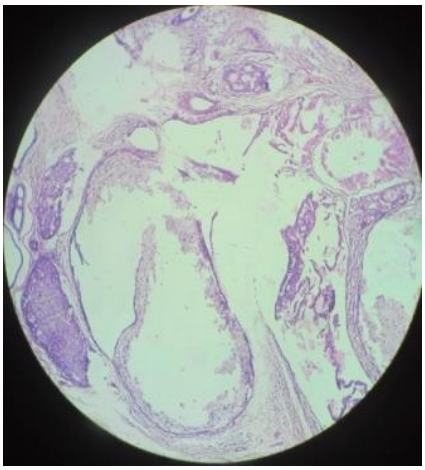

Figure-1.2: FCD with apocrine change, foamy macrophages and atypical ductal hyperplasia (ADH) with 10X magnification

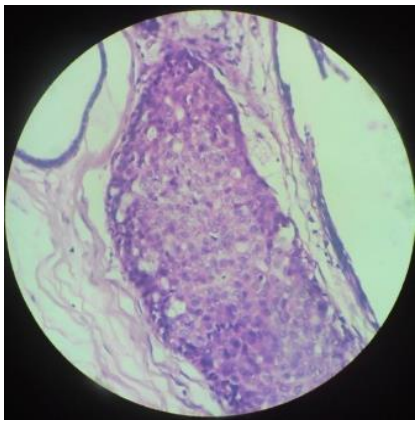

Figure-1.3: ADH a sheet of cells filled in a dilated duct with 40X Magnification of the same Image

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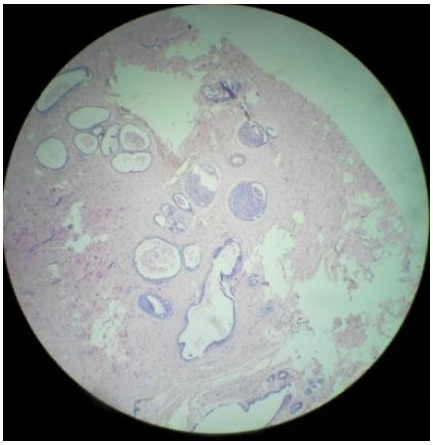

Figure-2.1: FCD of breast with dilated ducts with secretions in lumen, some ducts are dilated and completely filled with epithelial cells with 4X magnification

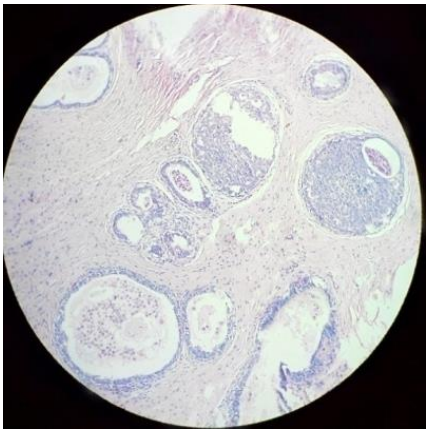

Figure-2.2: FCD of breast with dilated ducts with secretions in lumen, some ducts are dilated and completely filled with atypical epithelial cells and foamy macrophages (ADH) with 10X magnification

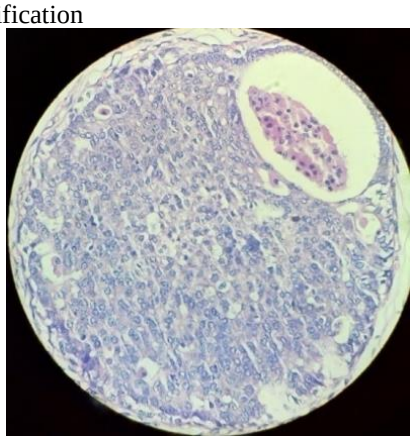

Figure-2.3: FCD of breast with ADH-Dilated duct completely filled with atypical epithelial cells and foamy macrophages (ADH) with 40X magnification of the same Image.

The above shown breast cancer images are collected from two different patents with different magnifications. Figure 1.1 to 1.3 tissues is affected by Fibrocystic Disease and atypical ductal hyperplasia a sheet of cells filled in a dilated duct shown in different magnified images. Figure-2.1 to 2.3 FCD of breast with dilated ducts with secretions in lumen, some ducts are dilated and completely filled with epithelial cells and FCD of breast with ADH-Dilated duct completely filled with atypical epithelial cells and foamy macrophages (ADH) with different magnification.

Staining methods routinely used in pathology lead to similar colour distributions in the biologically different regions of histo-pathological images. This causes problems in image segmentation for the quantitative analysis and detection of cancer. To overcome this problem, unlike previous methods that use pixel distributions, we propose a new homogeneity measure based on the distribution of the objects that we define to represent tissue components. Using this measure, we demonstrate a new object-oriented segmentation algorithm. Working with colon biopsy images, we show that this algorithm segments the cancerous and normal regions with 94.89 percent accuracy on the average and significantly improves the segmentation accuracy compared to its pixel-based counterpart.[13]

III. DIGITAL IMAGE PROCESSING

Image segmentation has a promising future as the universal segmentation algorithm and has become the focus of contemporary research. In spite of several decades of research up to now to the knowledge of authors, there is no universally accepted method for image segmentation, as the result of image segmentation is affected by lots of factors, such as: homogeneity of images, spatial characteristics of the image continuity, texture, image content. Thus there is no single method which can be considered good for neither all type of images nor all methods equally good for a particular type of image. Due to the above factors, segmentation remains a challenging problem in image processing and computer vision and continues to carry interesting research all over the world [1, 2].

Image Processing is made up of three layers mainly upper layer as Image Understanding, Middle layer as Image Analysis, Lower layer as Image Processing, as shown n figure 1. Image segmentation is the first step and also one of the most difficult tasks in image analysis. The objective analysis is for extracting information that is represented in the form of data from image via image segmentation, feature measurement and object representation as shown in figure 3. The result of image segmentation considerably depends on the accuracy of features measurement [3].

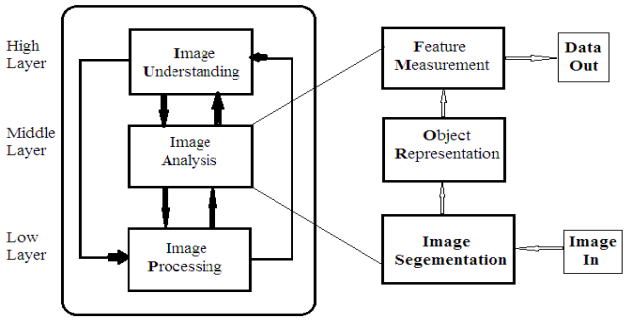

Figure 3: Image Engineering And Image Segmentation [3].

For the purpose of analyzing gastric colour pathologic cell images, a novel method is developed with gray-scale edge detection of mathematical morphology in this study. In combination with texture features of the image under investigation, this paper works on edge detection with various structuring elements (SEs) and gray-scale values.

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The results of the experiment are presented, and we found several advantages by using the morphological edge detection scheme for the analysis of gastric tumour pathologic cell images. [7]

Similar to other malignant tumour cells, gastric tumour pathologic cells could be recognized according to several representative characteristics as follows: (1) the volumes of the nuclei expand to 5–10 times as big as the normal ones; (2) the shapes of the cells are irregular, while the normal ones are usually circular, elliptical or rod-shaped; (3) the chromatin of the cells would increase and conglomerations would appear, while the chromatin in normal nuclei are homogeneous; (4) the nucleolus envelopes of the cells are obviously thicker than normal ones; (5) the nucleolus splits increase resulting in prominent tumour cellular proliferation; (6) cytoplasm decreases in the cells, therefore, the ratios of the area of the nucleus to the area of the cytoplasm increase. Sometimes, it is difficult to distinguish the benign tumour from malignant ones.

With the aid of image processing methods such as mathematical morphology which define the edge of the images, it becomes easier to identify the sizes, shapes and characteristics of pathologic cell images [8,9]. The edges of an image always include inherent information (such as direction, step character, shape, etc.), which are significant attributes for extracting features in image recognition.

In most cases, pixels along an edge change gradually, whereas those perpendicular to the direction of the edge usually have much sharper changes. Generally speaking, arithmetic for edge extraction is to detect whether mathematical operators of the pixels are coincident with the features of the edge. Additionally, edge based segmentation methods can extract the points at the boundary and then reconstruct the figures of segmentation regions, as discussed in Refs. [10,11].

Assorted approaches can be used to recognize gastric tumour pathologic cells. Among these measures, gray-scale edge detection by mathematical morphology is a new one. This method detects the investigated image with a structuring element (SE), examining if the SE can be well filled in the image, and checking whether the method of filling the SE is effective. By marking the position where the SE is filled in the image, the information of the image, which is related to the size and the configuration of the SE, can be attained. Therefore, by constructing various SEs, different image analysis can be performed and different structural information can be obtained. In order to achieve gray-scale edge detection for images of gastric tumour pathologic cells, gray morphological theories are applied, which are adjusted based on tumour cellular features and investigated to obtain image texture information [12]. We also performed simulation studies, in which various SEs are constructed and analyzed to compare with the experimental results.

Despite this modality being invasive, which is unpleasant for patients, physicians usually require a biopsy for a definite answer if they are suspicious about a certain abnormality in an image acquired by a non-invasive imaging modality. A closer view of the histopathological specimens can assist in verifying the colour type. Pathologists have been using microscopic images to study tissue biopsies for a long time, relying on their personal experience on giving decisions on the healthiness state of the examined biopsy. This includes distinguishing normal from abnormal (i.e. cancerous) tissue, benign versus malignant colours and identifying the level of colour malignancy. Nevertheless, variability in the reported diagnosis may still occur [33–35], which could be due but not limited to the heterogeneous nature of the diseases (i.e. not all samples referring to a certain colour subtype look identical, raising the issue of misclassification); ambiguity caused by nuclei overlapping; noise arising from the staining process of the tissue samples; intra-observer variability, i.e. pathologist not being able to give the same reading of the same image at more than one occasion; and inter-observer variability, i.e. increase in classification variation between different pathologists.

Therefore, through the past three decades, quantitative techniques have been developed for computer- aided diagnosis, which aim to assist pathologists in the process of cancer diagnosis [36]. Currently, the challenge remains in developing a better technique that not just automates the diagnostic procedure, but also applies the optimum texture feature extraction that better captures and understands the underlying physiology to improve cancer recognition accuracy.

A number of research studies have been applied to histopathological images for different colours in an attempt to automate the diagnosis procedure. Some of them relied on using one texture measure (i.e. method) for feature extraction, such as extraction of wavelet-based features [37–39], or using other measures individually like fractal dimension or grey-level co- occurrence matrix for classification [40,41]. Using more than one measure for classification was applied as well, such as using spatial and frequency texture features for classification by regression trees analysis [42]. Some used morphological characteristics for feature extraction [43,44], and others focused more on classifier improvement [45,46]. Regarding meningiomas, some used unsupervised learning techniques for training artificial neural networks, e.g. a self organising map, for classifying meningioma features derived by wavelet packet (WP) transform [37]. An average accuracy of 79% was reported for classifying four different meningioma subtypes. Others applied a supervised learning method for classification of meningioma cells [47], using a decision tree after selecting the most relevant features from a base of grey and coloured image features. Also in another two studies the performance of features extracted from four meningioma subtypes using adaptive WP transform was compared to local binary patterns [48] and to co-occurrence methods [49].

The WP method gave the highest classification accuracy of 82.1% when features were classified via a support vector machine classifier after applying a principal component analysis for dimensionality reduction.

As there is very limited research in the literature on fully-automating meningioma classification with significant accuracy, this research sets out to provide a novel method that combines model and statistical-based texture measures in an endeavour to provide a better understanding on how they relate to the underling physiology. The aim is to improve the classification accuracy by integrating the RGB colour channels that better assists the morphological process in segmenting the colour structure with the best combination of texture features that best captures the characteristics of the examined case.

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One of the prerequisites to grading or diagnosis of disease in histopathology images is often the identification of certain histological structures such as lymphocytes, cancer nuclei, and glands. The presence, extent, size, shape, and other morphological appearance of these structures are important indicators for presence or severity of disease.

IV. CONCLUSIONS

Tissue Processing, Staining and Image capturing of Pathological Cancer Images are plays a magor role in the detecting of the cancer at early stage. Image processing will predict the stage of the cancer in the absence of medical expert.

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